

IN THE CLAIMS

This listing of claims replaces all prior versions, and listings, in this application.

1. (previously presented) A modified polypeptide having β -glycosidase activity, said polypeptide comprising an amino acid sequence selected from:

- (a) the amino acid sequence of SEQ ID NO:2 mutated with at least one or more mutations in an amino acid residue selected from the group consisting of W433, E432 and M439;
- (b) the amino acid sequence of a family 1 glycosyl hydrolase mutated at an amino acid residue corresponding to at least one of W433, E432 and M439 of SEQ ID NO:2; and
- (c) a variant of (a) having β -glycosidase activity and mutated with an amino acid residue corresponding to at least one of W433, E432 and M439 of SEQ ID NO:2, wherein said variant has at least 95% identity to SEQ ID NO:2 over the entire length of the sequence;

and said polypeptide having an optional mutation of a catalytic nucleophilic residue of the active site.

2. (original) The polypeptide according to claim 1 in which the mutation is selected to broaden the substrate specificity of the polypeptide compared to a polypeptide not so modified.

3. (original) The polypeptide according to claim 1, wherein the mutation is an amino acid substitution.

4. (previously presented) The polypeptide according to claim 1 in which the polypeptide comprises:

- (i) SEQ ID NO:2 having one or more of W433, E 432 and M439 substituted by cysteine, valine or alanine; or

- (ii) the amino acid sequence as defined in (b) or (c) having one or more of the amino acid residues corresponding to W433, E432 and M439 of SEQ ID NO:2 substituted by cysteine, valine or alanine.

5. (previously presented) A modified polypeptide having β -glycosidase activity, said polypeptide comprising an amino acid sequence selected from:

- (a) the amino acid sequence of SEQ ID NO:2 mutated with at least one or more mutations selected from the group consisting of W433C, E432C and M439C;
- (b) the amino acid sequence of a family 1 glycosyl hydrolase mutated in an amino acid residue corresponding to at least one of W433, E432 and M439 of SEQ ID NO:2 wherein the amino acid is substituted by a cysteine residue; and
- (c) a variant of (a) having β -glycosidase activity and mutated with an amino acid residue corresponding to at least one of W433, E432 and M439 of SEQ ID NO:2 wherein the amino acid is substituted by a cysteine residue and wherein said variant has at least 95% identity to SEQ ID NO:2 over the entire length of the sequence.

6. (previously presented) The polypeptide according to claim 5, wherein the cysteine residue introduced by the mutation is chemically modified.

7. (previously presented) The polypeptide according to claim 6, wherein the cysteine residue is modified so as to comprise a positively-charged group.

8. (original) The polypeptide according to claim 7, wherein the positively charged group is of formula $-(CH_2)_n-N^+R_3$, wherein n is a positive integer from 1 to 4 and each R, which may be the same or different, is H or a C₁-C₄ alkyl group.

9. (original) The polypeptide according to claim 8, wherein the positively charged group is $-CH_2CH_2NMe_3^+$.

10. (previously presented) The polypeptide according to claim 6, wherein the cysteine residue is modified so as to comprise a negatively-charged group.

11. (original) The polypeptide according to claim 10, wherein the negatively-charged group is of formula $-(CH_2)_n-SO_3^-$ or $-(CH_2)_n-COO^-$, wherein n is a positive integer from 1 to 4.

12. (original) The polypeptide according to claim 11, wherein the negatively-charged group is of formula $-(CH_2)_n-SO_3^-$.

13. (previously presented) The polypeptide according to claim 6, wherein the cysteine residue is modified so as to comprise an uncharged group.

14. (original) The polypeptide according to claim 13, wherein the uncharged group is a C_1 - C_4 alkyl group.

15. (original) The polypeptide according to claim 14, wherein the uncharged group is methyl.

16. (original) The polypeptide according to claim 1, which further comprises a mutation of a catalytic nucleophilic residue of the active site.

17. (previously presented) The polypeptide according to claim 16, wherein the further mutation is:

- (i) E387A or E387G in SEQ ID NO:2 or
- (ii) substitution with alanine or glycine at the amino acid residue corresponding to E387 of SEQ ID NO:2 in the amino acid sequence as defined in (b) or (c) of claim 1.

Claim 18 (canceled)

19. (original) The polypeptide according to claim 1, wherein the family 1 glycosyl hydrolase is *Sulfolobus solfataricus* β -glycosidase.

20. (original) The polypeptide according to claim 6, which further comprises a mutation of a catalytic nucleophilic residue of the active site.

21. (previously presented) The polypeptide according to claim 20, wherein the further mutation is:

- (i) E387A or E387G in SEQ ID NO:2 or
- (ii) substitution with alanine or glycine at the amino acid residue corresponding to E387 of SEQ ID NO:2 in the amino acid sequence as defined in (b) or (c) of claim 5.

Claim 22 (canceled)

23. (original) The polypeptide according to claim 6, wherein the family 1 glycosyl hydrolase is *Sulfolobus solfataricus* β -glycosidase.

Claims 24-26 (canceled)

27. (withdrawn) A method for hydrolysing a β -glycoside, which method comprises contacting a glycoside substrate with a modified polypeptide having β -glycosidase activity, said polypeptide comprising an amino acid sequence selected from:

- (a) the amino acid sequence of SEQ ID NO:2 mutated with at least one or more mutations in an amino acid residue selected from the group consisting of W433, E432 and M439;
- (b) the amino acid sequence of a family 1 glycosyl hydrolase mutated in an amino acid residue corresponding to at least one of W433, E432 and M439 of SEQ ID NO:2; and

- (c) a variant of (a) having β -glycosidase activity and mutated with an amino acid residue corresponding to at least one of W433, E432 and M439 of SEQ ID NO:2, wherein said variant has at least 95% identity to SEQ ID NO:2 over the entire length of the sequence;
- and said polypeptide having an optional mutation of a catalytic nucleophilic residue of the active site.

28. (withdrawn) The method according to claim 27, wherein the glycoside substrate is selected from the group consisting of a glucoside, a galactoside, a fucoside, a xyloside, a mannoside, and a glucuronide.

29. (withdrawn) The method according to claim 27, wherein the polypeptide is contacted with a sample containing at least two different glycosides.

30. (withdrawn) A method for hydrolysing a β -glycoside, which method comprises contacting a glycoside substrate with a modified polypeptide having β -glycosidase activity, said polypeptide comprising an amino acid sequence selected from:

- (a) the amino acid sequence of SEQ ID NO:2 mutated with at least one or more mutations selected from the group consisting of W433C, E432C and M439C;
- (b) the amino acid sequence of a family 1 glycosyl hydrolase mutated in an amino acid corresponding to at least one of W433, E432 and M439 of SEQ ID NO:2 wherein the amino acid is substituted by a cysteine residue; and
- (c) a variant of (a) having β -glycosidase activity and mutated with an amino acid residue corresponding to at least one of W433, E432 and M439 of SEQ ID NO:2 wherein the amino acid is substituted by a cysteine residue, wherein said variant has at least 95% identity to SEQ ID NO:2 over the entire length of the sequence;
- wherein the cysteine residue introduced by the mutation of (a), (b) or (c) is chemically modified.

31. (withdrawn) The method according to claim 30, wherein the glycoside substrate is selected from the group consisting of a glucoside, a galactoside, a fucoside, a xyloside, a mannoside, and a glucuronide.

32. (withdrawn) The method according to claim 30, wherein the polypeptide is contacted with a sample containing at least two different glycosides.

Claims 33-37 (canceled)

38. (previously presented) The polypeptide according to claim 1, wherein the variant (c) has at least 99% identity to SEQ ID NO:2 over the entire length of the sequence.

39. (previously presented) The polypeptide according to claim 1, said polypeptide comprising the amino acid sequence of a family 1 glycosyl hydrolase mutated in an amino acid residue corresponding to at least one of W433, E432 and M439 of SEQ ID NO:2.

40. (previously presented) The polypeptide according to claim 39 wherein said mutation consists of substitution of the amino acid residue corresponding to at least one of W433, E432 and M439 of SEQ ID NO: 2 by cysteine, valine or alanine.

41. (previously presented) The polypeptide according to claim 1, said polypeptide comprising an amino acid sequence selected from:

- (a) the amino acid sequence of SEQ ID NO:2 mutated with at least one or more mutations in an amino acid residue selected from the group consisting of W433, E432 and M439; and
- (b) the amino acid sequence of a family 1 glycosyl hydrolase mutated at an amino acid residue corresponding to at least one of W433, E432 and M439 of SEQ ID NO:2;

wherein said polypeptide further comprises a mutation of a catalytic nucleophilic residue of the active site.

42. (previously presented) The polypeptide according to claim 1 having β -glycosidase activity, and comprising an amino acid sequence having at least 95% identity to SEQ ID NO:2 over the entire length of the sequence and a mutation in an amino acid residue corresponding to at least one of W433, E432 and M439 of SEQ ID NO:2.

43. (previously presented) The polypeptide according to claim 5 having β -glycosidase activity, and comprising an amino acid sequence having at least 95% identity to SEQ ID NO:2 over the entire length of the sequence and a mutation in an amino acid residue corresponding to at least one of W433, E432 and M439 of SEQ ID NO:2 wherein the amino acid is substituted by a cysteine residue.

44. (withdrawn) The method according to claim 27, wherein said polypeptide has β -glycosidase activity, and comprising an amino acid sequence having at least 95% identity to SEQ ID NO:2 over the entire length of the sequence and a mutation in an amino acid residue corresponding to at least one of W433, E432 and M439 of SEQ ID NO:2.

45. (withdrawn) The method according to claim 30, wherein said polypeptide has β -glycosidase activity, and comprising an amino acid sequence having at least 95% identity to SEQ ID NO:2 over the entire length of the sequence and a mutation in an amino acid residue corresponding to at least one of W433, E432 and M439 of SEQ ID NO:2 wherein the amino acid is substituted by a cysteine residue.